BIOLOGIA PLANTARUM (PRAHA)

7 (3) : 199—211, 1965

Nuclear Instability and Chromosomal Mosaicism in the Polyploids of *Trigonella foenum-graecum*

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Received October 27, 1964

Abstract. It was observed that the polyploids of *Trigonella foenum-graecum* produced by seed treatment with 0·2% colchicine died after two cotyledonary stage, while the ones produced by seedling treatment survived. The root and shoot of seed treated plants were found to be mixoploids. The root tips exhibited 2n to 12n chromosomes and with the passage of time there is a regular decrease in the frequency of cells with lower chromosomes. The frequency of dividing cells decreases considerably towards the end of the 6th day due to the highploidy of most of the cells. Disturbance of nuclear cytoplasm ratio may be responsible for nuclear instability of polyploid plants. In case of seedling treatment the first formed tissue was mixoploid of high level similar to that of seed treated ones but in some cases the growing tip reverted to low level of ploidy which lead to more or less normal growth.

Since the difference in the constitution of seed and seedling treated plants lies in their root system, it has been suggested that probably highploidy of root system in the former may be responsible for the 100% mortality of plants.

The inconsistency or instability of chromosome number within an individual is no longer considered to be rare. Many workers have reported this phenomena in the plant kingdom (Hegwood and Hough 1958, Love 1938). The chromosome mosacism has been reported in animals like salamanders (Fankhauser 1945). In human tissue many subdiploid cells have been observed (Therman and Timonen 1951). The present report is based on the inconsistency of chromosome number observed on colchicine induced polyploids of *Trigonella foenum-graecum*.

The polyploids of *Trigonella foenum-graecum* were artificially induced by seed as well as seedling treatment. It was noted that the seed-treated plants (polyploids produced by seed treatment) did not grow beyond the two cotyledon stage and in due course died. Since the shoot-treated plants continued to live, it was thought that perhaps the cause of the high mortality in the seed treated plants lies in the polyploid roots. It may be mentioned that all the seeds after treatment with 0·2% colchicine produced polyploid plants while this was not the case in shoot-treated ones. A comparative study of seed and seedling-treated plants was undertaken to investigate the morpho-

logical and chromosomal changes in the polyploids produced by the two methods with the ultimate view of finding the causes of the 100% mortality in the seed-treated polyploids.

Materials and Methods

The plants used in the present study were grown in an enclosure in the Lucknow University campus during the years 1961—63. Seeds as well as seedlings were subjected to colchicine treatment. The most suitable strength of aqueous colchicine solution was determined by trials. For seed treatment seeds were soaked in 0·2% solution of colchicine for 4, 8, 12 and 24 hours beyond which no germination took place. For each duration of treatment 300 seeds were taken and after washing in distilled water 12 seeds were sown in each pot along with suitable controls which were kept moist in distilled water for the same duration. Since the germination happens to be very quick, the seeds were taken out after every twenty-four hours for six consecutive days. Root tips were fixed in acetic alcohol (1 : 3). For morphological studies root as well as shoot were measured daily in order to find the root: shoot growth ratio. As it was noticed that after the 6th day the cells of the root tip became flaccid and degeneration began, cytological studies of somatic chromosomes could not be carried further. However, the morphological study was continued until the 24th day.

In the second part of the experiment the two-leaf seedlings were treated at their apical growing points by covering them with cotton wads and keeping them moist with 0·1%, 0·2% and 0·3% aqueous colchicine. The duration of treatment was 6, 12 and 24 hours continuously, coupled with a set which included treatment of 8 hours daily for 1, 2 and 3 days. This method ultimately resulted in 8, 16 and 24 hours of intermittent treatment. This was done in order to subject the meristematic cells to colchicine during the period when somatic division took place. Out of 600 plants thus treated, 99 proved to be variants with morphologically as well as cytologically changed characters. Most of these died before reaching maturity and a few that survived showed to have reverted to diploidy or lower level of mixoploidy on cytological examination.

The root tips for chromosomal study were fixed in acetic alcohol (1 : 3) for 24 hours, then heated in a 1 : 9 mixture of N-HCl and acetocarmine, cooled and mounted in a drop of acetocarmine. Another method that gave good result was heating the root tip in N-HCl after fixation in acetic alcohol followed by washing in distilled water. These were again placed in acetic alcohol for five minutes, then heated in acetocarmine till steaming. The use of acetic-alcohol after HCl hydrolysis increases stainability of somatic chromosomes.

Results

For the sake of convenience the morphological and cytological features will be taken up separately.

Morphological features

In the seed-treated plants development did not proceed beyond the two-cotyledon stage. The cotyledons were more horizontally placed than in con-

trols. They were thicker and coarse; the apical dome was broader than in the controls (Figs. 19, 21) and later on it disintegrated without producing any leaves. Table 1 brings out the following points clearly. 1. Diameter of root increased up to the 15th day and then decreased, probably due to disintegration of cells inside, while that of control did not show any change. 2. Diameter of hypocotyl decreased with age in the control as well as in the treated. 3. Length and breadth of cotyledons continued increasing with age although the

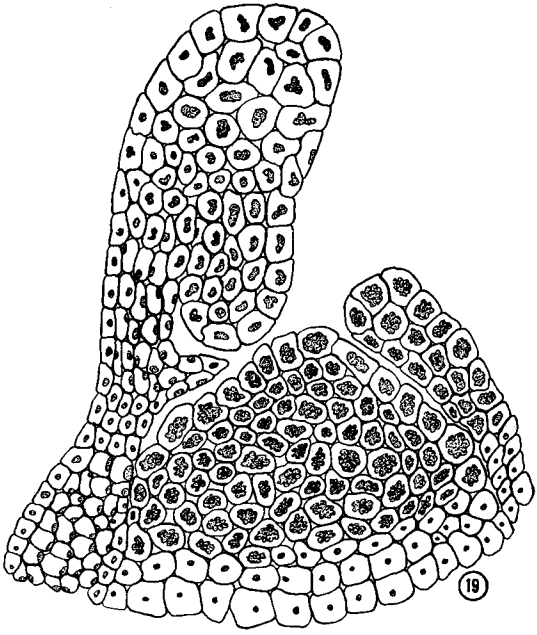

Fig. 19. Median longitudinal section of high ploid shoot-apex. Note the large lobed nuclei in static condition.

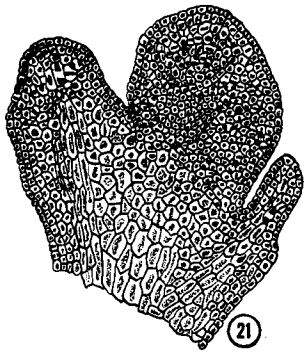

Fig. 21. Median longitudinal section of control shoot apex showing actively dividing cells. Magnification: — Figs. 19 and 21 × 150; fig. 20 × 740.

size of the treated was smaller than that of control. The disintegration began with root and proceeded upwards. Obviously on the 20th day only the root showed disintegration, while hypocotyl and cotyledons were not adversely effected. They died only when the root had completely disintegrated. Since the seed treated polyploids did not show difference in the size among themselves with the duration of treatments, the observations have been condensed by taking the mean. Although references can be cited where polyploidy has resulted in larger cotyledons (KRYTHE and WELLENSICK 1942, TANIGAWA and CHURO 1953), we observed a decrease in length and breadth while there was a considerable increase in the thickness of the treated cotyledons in comparison with the control. In Fig. 1 the comparative growth rate of the seed treated plants is shown up to the 24th day where disintegration had already set in the root tip region while in some cases it was prominent in the intermediate region between the root and the hypocotyl. Note the control of same age which has developed secondary roots, nodules, as well as leaves. After

15 days the majority of plants which remained alive up to the 24th day showed development of primary and secondary roots (Fig. 2). On cytological examination these were found to be the cases of reversion to diploidy, thereby proving the polyploid nature of the root to be fatal for the survival of the plant.

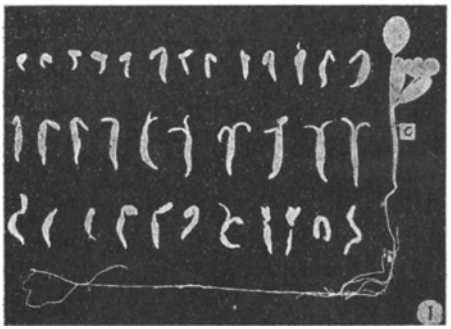

Fig. 1. The first and second rows show growth colchicine treated seeds up to 23rd day, while the third row shows various stages of disintegration of seedlings. The control (C), which is 23 days old, clearly shows secondary roots and nodules. $^1/_2$ natural size.

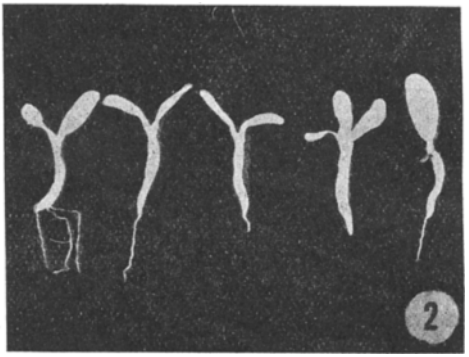

Fig. 2. Shows reversion from high level mixoploid roots to diploidy. Mark the thin diploid roots coming out of mixoploid tissue. The second seedling from right shows three cotyledons. Natural size.

The newly developed diploid roots (Fig. 2) may have continued to grow because they appeared quite healthy but the apex of shoot failed to revert to diploid level or continue growth in its mixoploid condition. So in the absence of any growth of the shoot the food material could not be synthesized and the plant ultimately died.

Nodule formation took place only in the roots of controls (Fig. 1), while seed-treated roots having mixoploid tissue or even in cases of reversion to diploidy, nodule formation did not take place.

The shoot and root growth ratio was well marked in control and seed-treated plants. The data of the growth distribution of root and shoot is represented in Fig. I. by plotting the shoot/root ratio against the days. On the first day the value was zero, since no shoot development has taken place. On the 2nd day maximum was reached in 24-hour treatment while the minimum in the case of 8-hour treatment. On the 3rd day values in all the treated plants were overlapping. On the 6th day maximum was reached by 12-hour treatment. While in the treated plants growth was more in the shoot region than in the root, in the control reverse was the case. The root grew at a rate which was twice that of the shoot. The graph also shows that all the timings of the treatment have been effective enough to show the effect of the chemical.

The structure of stomata was normal (Fig. 3) in the cotyledons of controls, while in the seed-treated plants there was only formation of the stomatal mother cell which in most cases failed to divide to produce two normal guard cells (Fig. 4). However, in 0·5% of the stomata observed after 4-hour and

24-hour treatment two guard cells were formed but they lacked the thickenings on the inner side facing the aperture and hence do not function in the normal fashion. Table 2 gives the comparative incidence of stomatal mother cells per field and its relative size after the 4, 8, 12 and 24-hour treatments. It will be noticed that the maximum number of stomatal mother cells per field was after the 12 hours' treatment and the minimum in the 4 hours. Such figures do not give any correlation with the duration of treatment but comparison of the size of the stomatal mother cell revealed that the 12-hour treated seeds had the highest number because they had the smallest size of stomatal mother cells. Moreover, the 4-hour treated seeds had the minimum numbers per field, as they had the largest size of stomatal mother cells. One of the interesting differences observed in the anatomy of these plants was in the development of the tracheids in the control and the seed-treated. In the seed-treated the first formed tracheids of the roots had only reticulate ornamentation (Fig. 5), while in control spiral and annular tracheids developed first (Fig. 6), later accompanied by reticulate tracheids. In the treated plants vascular elements made their appearance in the root tips on the 3rd, day while in the control on the sixth day. It has been reported earlier that where elongation of the plant organ is retarded or inhibited, pitted elements appear close to the apical meristem (GOODWIN 1942, SMITH and KRYTHE 1942). There is also evidence among naturally growing roots that those which elongate much have a larger proportion of extensible form of xylem cells than roots showing little elongation (SCHERER 1904). In the seed-treated cotyledons the bulk of vascular elements was greater than in the control. There was more spongy parenchyma than in the control which gave extra thickness to the seed treated cotyledons.

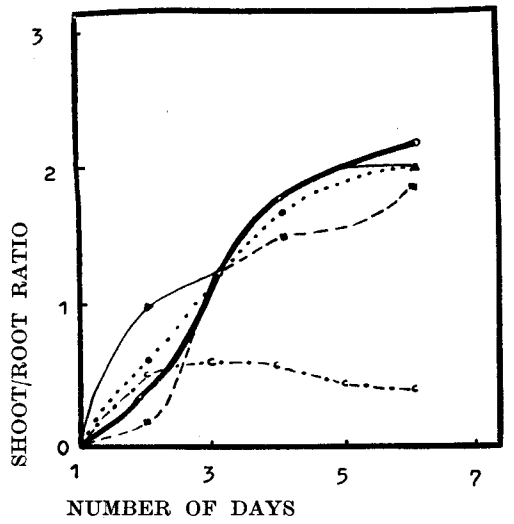

Fig. I. Graphic representation of shoot root ratio versus the number of days after treatment.

Cytology

The somatic studies of root tips of seed-treated polyploids were carried out for all the four sets of treatment after every 24 hours. These studies revealed that all the root tips were mixoploids with varying proportion of cells having a different number of chromosomes. These cells generally exhibited 2n to 12n

Table 1

Comparison of the morphological characters of control and mixoploids

Serial No.	Age of the plant days	Treated or control	Diameter of root mm.	Diameter of hypocotyl mm.	Length of cotyledon mm.	Breadth of cotyledon mm.
I	6	Treated	1·66	4·0	5·2	2·4
		Control	0·52	1·68	9·6	4·2
II	10	Treated	1·9	3·0	6·5	3·0
		Control	0·5	1·5	15·0	6·5
III	15	Treated	2·0	3·0	7·0	3·4
		Control	0·5	1·3	12·3	6·0
IV	20	Treated	0·7	2·4	10·6	4·0
		Control	0·5	1·1	15·5	7·5

chromosomes in different proportions of the mixoploid condition. The highest number of countable chromosomes was 14n. Another interesting fact was the extreme reduction in size that accompanied the increase in chromosome number (compare Figs. 12 and 18).

The mixoploid condition of root tip cells is clearly seen in Fig. 10. Figure 9 shows a highploid cell. Three groups of 48, 48 and 16 chromosomes are seen in Figure 8. A few cells had double spindles (Figs. 7 and 11). This may indicate delay in the formation of the cell wall between two newly produced cells in the preceding cell division. The mixoploid assemblage of cells is shown in figures 13—17. These show $2n + 4 = 20$ (Fig. 13); $4n + 2 = 34$ (Fig. 14); $6n = 48$ (Fig. 15); $8n = 64$ (Fig. 16) and $10n = 80$ (Fig. 17). Figure 18 shows a cell with the high chromosome number of approximately 350 distributed into unequal groups of about 200 and 150 chromosomes. This cell was exceptionally large.

The percentage of cells with different chromosome numbers as they appeared in the study of six-day samples of roots of treated plants are given

Table 2

Comparative data of the frequency of stomatal mother cell in relation to its size in the 4, 8, 12 and 24 Hr. treated seed plants

Duration of treatment	4 Hr.	8 Hr.	12 Hr.	24 Hr.
No. of stomatal mother cells per field	15	16	26	17
Size of stomatal mother cell	22·1 μ	20·8 μ	18·2 μ	19·5 μ

in Table 3. An examination of this table brings out the following points clearly. 1. On the first day diploid cells were present in fairly high frequency. The octaploid cells appear only in 3·3% of the cells in 4-hour treatment. 2. With the passage of time, the diploid cells went on decreasing at a rapid rate with almost complete disappearance on the 5th day, while the high ploid cells continued to increase in an inverse proportion. 3. The maximum number of tetraploid cells was observed on the 3rd day. The cells which had attained tetraploidy by further action of the chemical gave rise to octaploid cells with the result that the tetraploids also started diminishing, while on the 5th day there was an increase in the octaploids reaching a maximum on the sixth day. On the fifth day the tetraploids formed only a minority of the total number cells of, while the high-ploids dominated. The 12n number appeared for the first time on the fourth day. The number of 8n cells increased continually. It will be noticed that since the level of ploidy reached was very high towards the fifth day, accompanied by a decrease in the frequency of metaphase plates (Fig. II), it proves that highploid cells did not undergo normal division and since division almost ceased towards the 6th day, disintegration set in leading to the death of the tissue.

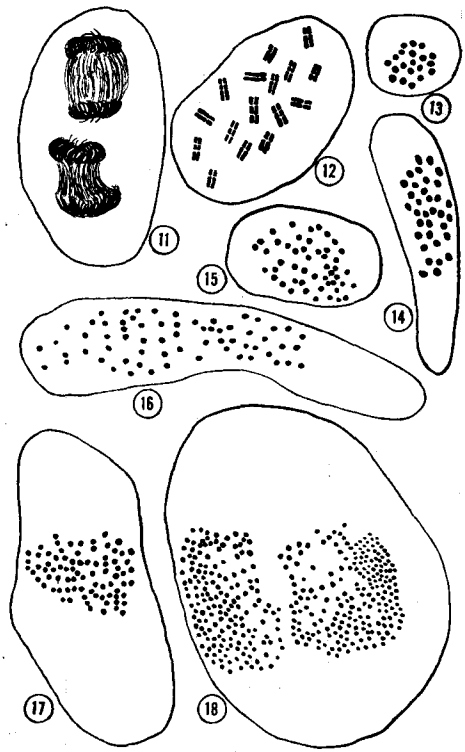

Fig. 11. A cell with double spindles showing spindle fibres.
Fig. 12. Somatic chromosomes of control.
Figs. 13—17. Mixoploid cells showing 2n + 4 = 20 (fig. 13); 4n + + 2 = 34 (fig. 14); 6n = 48 (fig. 15); 8n = 64 (fig. 16); 10n = 80 (fig. 17).
Fig. 18. A cell with two groups of about 200 and 150 chromosomes. Magnification: — Figs. 11—18 × 740.

The colchicine treatment caused continual multiplication of chromosomes in *Trigonella*, it did not stop at tetraploid level ultimately reaching 12n. Such highploidy became fatal for the plant. Further growth of both root and shoot was possible only if somehow there was reversion to diploidy or mixoploidy of lower level. The hypo and hyperploid cells (Figs. 13, 14) may have arisen by unequal separation at anaphase forming two cells in which one gains at the expense of the other.

The shoot tips and leaf tips were also squashed to determine their chromosomal constitution. The tissue of both these organs was mixoploid like root tips and here also the degree of ploidy reached such a high level that it obviously upset the physiological balance so much that cells did not survive. The very marked effect of this mixoploid tissue was seen on the shoot tip

Table 3

Frequency (%) of the mixoploid cells in the 4, 8, 12 and 24 hrs. treatment on first 6 days after treatment

Level of n.	DAYS					
	1st.	2nd.	3rd.	4th.	5th.	6th.
24 Hr.						
2 n		52·4	26·6	1·5		
3 n		2·8				
4 n		42·8	73·4	50·2		16·6
8 n				32·8		49·2
10 n				12·5		33·2
12 n				3		
12 Hr.						
2 n	60	9·6	8·4	4·6		
3 n		4·8				
4 n	40	85·4	74·5	65·6	32·3	33·3
8 n			16·9	29·6	50·0	66·6
10 n					17.6	
8 Hr.						
2 n	40·4	14·7	4·0	1·4		
4 n	59·6	82·3	63·0	58.8	23·8	32·2
6 n			9·0			
8 n		2·9	24·0	35·2	38·0	58·0
10 n				4·4	23·8	6·4
12 n					14·2	3·2
4 Hr.						
2 n	35·4	9·4	40·7	4·7	4·1	
3 n		1·3				
4 n	61·2	83·7	59·2	49·4	39·0	31·1
6 n		1·3				
8 n	3·3	4·1		37·6	38·0	55·5
10 n				7·0	12·5	13·3
12 n				1·1	6·2	

where there was no further growth of the tissue, while in the case of the controls normal growth of shoot took place. For studying the exact condition of shoot tip, median longitudinal sections of 21 days old seed treated shoot apices were cut. The apical dome of highploids was broader (Fig. 19) than the diploid (Fig. 21), which is normally the condition of the apical meristem

when the apex is in the resting stage. The cells of treated tips had deeply stained homogeneous nuclei. The cells were hypertrophied and remained inactive. In a few places, areas of disintegration were observed which resulted

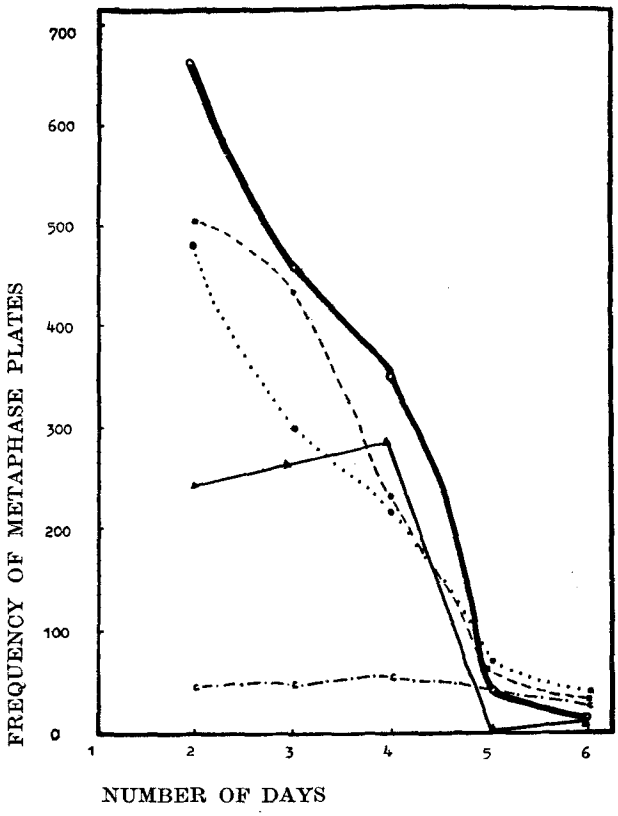

Fig. II. Accumulation of metaphase plates in the root tips after different treatments on 1—6 day.

due to death of highploid cells. In these cells the walls were densely stained, the cytoplasm was plasmolysed and in some a large number of vacuoles were present accompanied by a large number of nucleoli, which is indirect proof of high ploidy of the cells. In contrast to the seed-treated seedlings in the controls there was an abundance of karyokinetic activity. However, another point of great interest was the presence of starch grains (Fig. 20) in the cells of the hypocotyl just below the apex in the case of seed-treated seedlings,

whereas none were seen in the control. The starch grains showed characteristic crosses traversing their body which divided them in twos, threes, fours and eights, analogous to the arrangement of spores in the sporad.

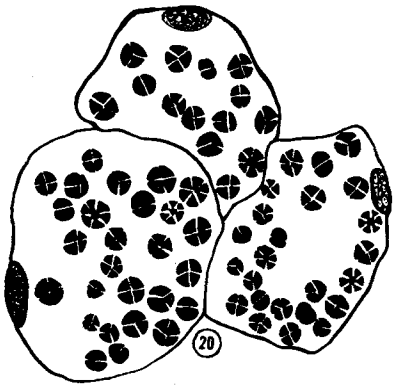

Fig. 20. Cells of the hypocotyl of highploid showing starch grains.

Discussion

The present investigation on the chromosome instability in highploid *Trigonella foenum-graecum* supply further evidence for a complex cytological mechanism characteristic of colchiploids. The highploid *Trigonella* induced by colchicine treatment are unstable and exhibit more or less conspicuous chromosomal mosacism in their somatic tissue.

With regard to the cause of variation of chromosome numbers, in the present study it is postulated that the colchicine treatment somehow brings about a loss of control on the division cycle and the chromosomes continue to divide but do not separate to form distinct nuclei. This process is repeated until the number 12n is reached. Such high chromosome number upsets the normal physiological processes so much that ultimately cell metabolism stopped functioning and disintegration set in.

The present study has lent weight to the fact that chromosome mosacism is seen generally in highploids. This may suggest that a highploid condition has a certain causal relation to mosacism. This is also inferred from the fact that hitherto chromosome mosacism has been reported quite frequently in polyploid plants with chromosome numbers ranging from 4n to 8n (BRITTON and HULL 1959, 1957; BROWN 1951; MENZEL 1952; PARTHSARATHY 1951, VAARAMA 1949a & b; O'MARA 1942, LOVE 1938). Disturbance of nuclear cytoplasmic relationship may be responsible for nuclear instability in polyploid plants. On the other hand, nuclear abnormalities caused by various means of artificial treatment show that disturbance of metabolic process is highly responsible for such phenomena. The latter, however, may arise from most varied causes, including gene actions, actions of certain metabolic products, the influence of various external factors etc. The relationship between metabolism and mutations in plants was discussed by D'AMATO and HOFFMAN-OSTERNHOF (1956) in a comprehensive review. According to HUSKINS et al. (1948, 1950) it is plausable that the increase of mosacism with increasing chromosome number and genome complexity may be due to progressively

greater derangement of the controlling physiological processes. NĚMEC (1962) suggested that in mixoploid root tips highly polyploid cells may act as foreign bodies which can be prevented from growing and differentiating; this may be the cause of lack of development of shoot and root tips in seed treated polyploids.

It is interesting to speculate on the factors which inhibit the growth of the shoot and root with the result that no further development takes place after the two cotyledon stage. Mixoploid tissue has been observed in shoot tip as well as root tip. In cases where seedlings were treated with colchicine the first formed tissue was mostly mixoploid. The leaves that came out were dark coloured, distorted and thick. Their squash revealed the presence of mixoploid condition. The range of ploidy appeared to be on the same pattern as in root tips of the seed treated polyploids on later days. There is no doubt that some growth did take place after colchicine treatment but most of these plants ultimately died and those that survived were found on cytological examination to be diploids or mixoploids of lower level, definitely between 2n and 4n. On the contrary, shoot treatment of *Capsicum frutescens* resulted in the production of tetraploids only and no mixoploid tissue was met with (RAGHUVANSHI & JOSHI 1964). This clearly shows that highploidy is also fatal in shoot-treated polyploids of *Trigonella*, but in this case there are more chances of reversion to diploids or lowploid condition, while in the case of seed treated polyploids the shoot never showed any growth beyond the two cotyledon stage and ultimately died. One of the differences in the two cases was in the root system; the roots of seed treated seedling were mixoploids while those of seedling treated were normal diploids and quite healthy, performing all their functions. The mixoploid roots on the other hand grow only for a few days. In these roots the ploidy of newly formed cells continued to increase, ultimately reaching the 12n level and this happens to be fatal. There is no production of secondary root system, so in this case the root system obviously cannot supply nutrition to the shoot. Disintegration begins from the root tip and ultimately the hypocotyl and cotyledons also perish.

In the seed-treated plants where the root has reverted to diploidy without further growth of the shoot, the explanation may be that cells at the apex have attained maturity and are not in an active state of growth permitting division and production of the shoot. The highploidy may be responsible for this. The cells do not remain in an active state of growth throughout their life but there is a certain critical period, during which if proper growth conditions are met with they divide and build up the plant body. Since in treated seeds no further growth is met with, it is reasonable to postulate that the cells passed the period of active growth while the root was not efficient enough to meet the necessities of plant life and hence no further development of plant took place beyond the two cotyledon stage.

Further evidence for the postulations put forward is provided by the examination of vertical sections of the shoot apex; in both the control and the seed treated plants (Figs. 21, 19). In the treated plants, the apical dome is broader than the diploid which is normally the condition of the apical meristem when the apex is in the resting stage. In the controls the apex has actively dividing cells giving rise to the primordia of the shoot, whereas in

the case of the seed-treated plants the cells of the apical zone are larger in size with larger nuclei in a static condition. None of the cells show any sign of mitotic activity. On the other hand, they resemble the cells of the mature tissue which has stopped undergoing division in the control plants.

A detailed account of the cytological behaviour of the mixoploids produced by seedling treatment along with their progeny will be published later.

Acknowledgement

The authors wish to express their sincere thanks to the authorities of the Botany Department in making available facilities for this work. Fellowship from The Council of Scientific and Industrial Research is duly acknowledged by the junior author (S.J.).

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S. S. Raghuvanshi a Sheila Joshi, Cytogenetická laboratoř Botanického ústavu university v Lucknow, Lucknow, Indie: **Jaderná nestálost a chromozomální mozaikovitost u polyploidů *Trigonella foenum-graecum*.** — Biol. Plant. **7** : 199—211, 1965.

Bylo zjištěno, že polyploidy *Trigonella foenum-gracecum* (Pískavice řecké seno), indukované působením 0,2 % colchicinu na semena, hynou ve stadiu děložních lístků, zatímco polyploidy, indukované působením na semenáčky, přežívají. Kořeny a nadzemní části rostlin, u kterých bylo působeno na semena, jsou mixoploidní. Buňky kořenových špiček mají 2n až 12n chromozómů a během doby dochází k pravidelnému snížení počtu buněk s nízkým počtem chromozómů. Počet dělících se buněk se značně snižuje ke konci 6. dne, což je způsobeno vysokým stupněm ploidie většiny buněk. Porušení poměru jádra a cytoplazmy může způsobit jadernou nestálost polyploidních rostlin. Při působení na klíční rostliny první vytvořené tkáně byly mixoploidní, ale v některých případech dochází v růstovém vrcholu ke snížení stupně ploidie, který vede k více nebo méně normálnímu růstu. Polyploidy, indukované působením na semena a na semenáčky, se liší v kořenovém systému. Proto se předpokládá, že vysoká ploidie v kořenech podmiňuje 100% letalitu polyploidů, indukovaných působením kolchicinu na semena.

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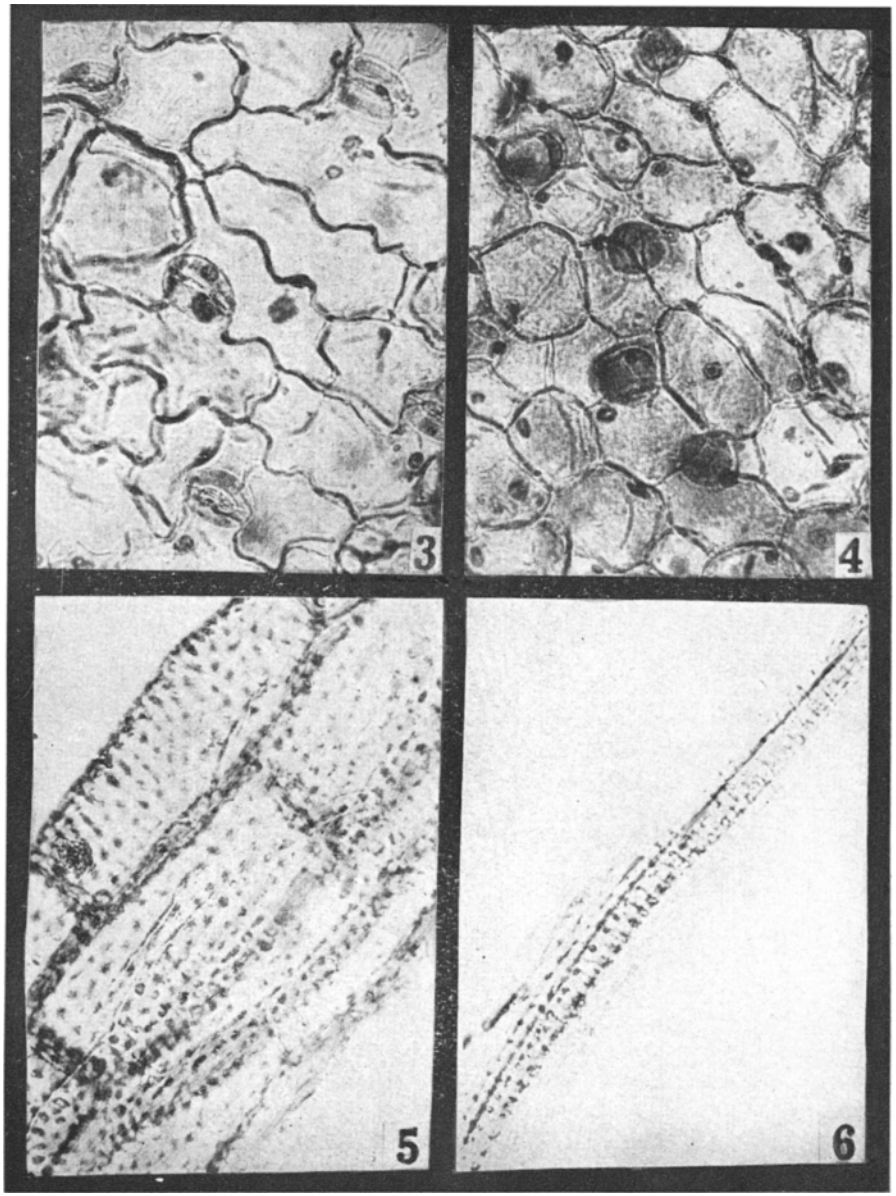
Установлено, что полиплоиды *Trigonella foenum-graecum*, индуцированные воздействием 0,2% колхицина на семена, гибнут в стадии семядольных листьев, между тем как полиплоиды, индуцированные воздействием на сеянцы, переживают. Корни и надземные части растений, у которых обрабатывались семена, миксоплоидны. Клетки корневых верхушек имеют 2 n—12 n хромосом и с течением времени закономерно снижается число клеток с низким числом хромосом. Число делящихся клеток значительно снижается к концу 6-го дня, что вызвано высокой степенью полиплоидии большинства клеток. Нарушение ядерно-цитоплазматического отношения может вызвать ядерную нестабильность полиплоидных растений. При обработке прорастающих растений первые образующиеся ткани были миксоплоидные, но в некоторых случаях происходит в верхушках роста снижение степени полиплоидии, приводящее к более или менее нормальному росту. Полиплоиды, индуцированные обработкой семяи или сеянцев, отличаются корневой системой. Поэтому предполагается, что высокая плоидность в корнях обуславливает 100% летальность полиплоидов, индуцированных действием колхицина на семена.



Figs. 3, 4. The peelings of lower epidermis of cotyledon of control and treated plants showing stomata and stomatal mother cells. Note also the difference in outline of epidermal cells.

Fig. 5. Reticulate ornamentation on the first formed tracheids of mixoploids.

Fig. 6. First formed spiral and annular tracheids in control. Magnification: — Figs. 3—6 × 1500

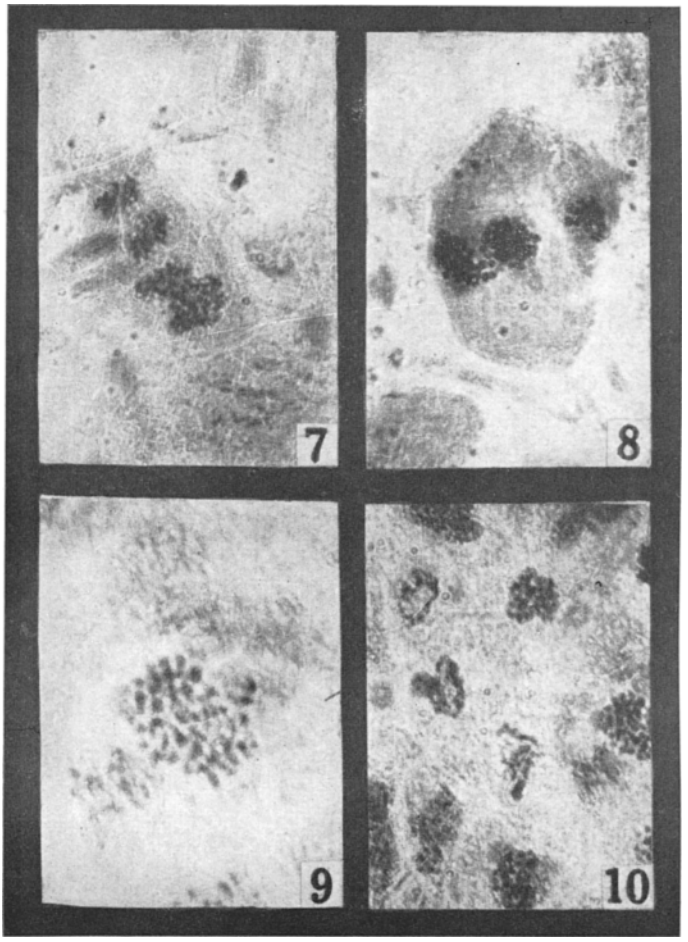


Fig. 7. A cell from the mixoploid root tip showing double spindles.
Fig. 8. Three groups of 48, 48 and 16 chromosomes in a single cell.
Fig. 9. A high ploid cell. Magnification: — Figs. 7—9 × 1080.
Fig. 10. Mixoploid condition of root tip. Magnification: — Fig. 10 × 300.